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Summary: Immunization of rabbits with a protein-free formulation consisting of liposomes containing 71% cholesterol and lipid A as an adjuvant induced antibodies that recognized highly purified nonoxidized crystalline cholesterol and rabbit VLDL/IDL by ELISA. In rabbits that were fed an atherogenic diet containing 0.5%-1.0% cholesterol, a marked diminution of hypercholesterolemia (as much as 979 mg/dl lower) was observed in the immunized animals when compared to nonimmunized controls. Analysis of aortic fatty streaks by automated morphometric probability-of-occurrence mapping of sudanophilia showed significantly diminished lesions in vaccinees in most areas of the aorta.

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Vaccination against cholesterol: immunologic modulation of diet-induced hypercholesterolemia and atherosclerosis in rabbits

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Abstract. Immunization of rabbits with a protein-free formulation consisting of liposomes containing 71% cholesterol and lipid A as an adjuvant induced antibodies that recognized highly purified nonoxidized crystalline cholesterol and rabbit VLDL/IDL by ELISA. In rabbits that were fed an atherogenic diet containing 0.5—1.0% cholesterol, a markedly lower hypercholesterolemia (as much as 979 mg/dl less) was observed in the immunized animals than in nonimmunized controls. Analysis of aortic fatty streaks by automated morphometric probability-of-occurrence mapping of sudanophilia showed significantly smaller lesions in vaccinees in most areas of the aorta.

Background

Cholesterol was first proposed in 1925 as an antigenic molecule against which specific antibodies could be induced in experimental animals in the presence of heterologous proteins that served as carriers [1]. The observation of apparent antibodies to cholesterol generated a considerable early interest in lipid immunology, and some controversy, which culminated more recently in the development of specific immunoassays for a wide range of steroid hormones (reviewed in [2]). In 1988, monoclonal antibodies to cholesterol were developed by immunization of mice with protein-free liposomes heavily loaded (71 mol%) with unconjugated highly purified nonoxidized cholesterol as an antigen and lipid A, the endotoxic portion of bacterial lipopolysaccharide, as an adjuvant [3]. The antibodies recognized purified, nonoxidized, crystalline cholesterol by ELISA and immunogold electron microscopy.

After development and validation of an ELISA for detecting antibodies to purified cholesterol, naturally occurring antibodies to cholesterol were found in sera from normal humans [4] and pigs, but not guinea pigs [5]. As shown in Fig. 1, using the ELISA technique with crystalline cholesterol as an antigen, we have now assayed sera from 742 preimmunization bleedings obtained from military personnel prior to testing of an unrelated vaccine. Every sample contained easily detectable antibodies to cholesterol, thus extending our previous studies that suggested that naturally occurring IgM and IgG antibodies to cholesterol are present in virtually all normal human sera [4].

The ubiquitous presence of naturally occurring antibodies to cholesterol in sera from young adults suggested the possibility that antibodies to cholesterol might play some role in the metabolic regulation of serum cholesterol, perhaps even a hormone-like role. Several early reports showed that immunization with heterologous β-lipoproteins [6], or a heterologous protein–cholesteryl ester antigen in which cholesteryl sebacate was esterified to a heterologous protein carrier [7,8], inhibited the development of diet-induced hypercholesterolemia and aortic atherosclerosis in rabbit and cockerel models. In the present study, to test whether antibodies produced against liposomal cholesterol might

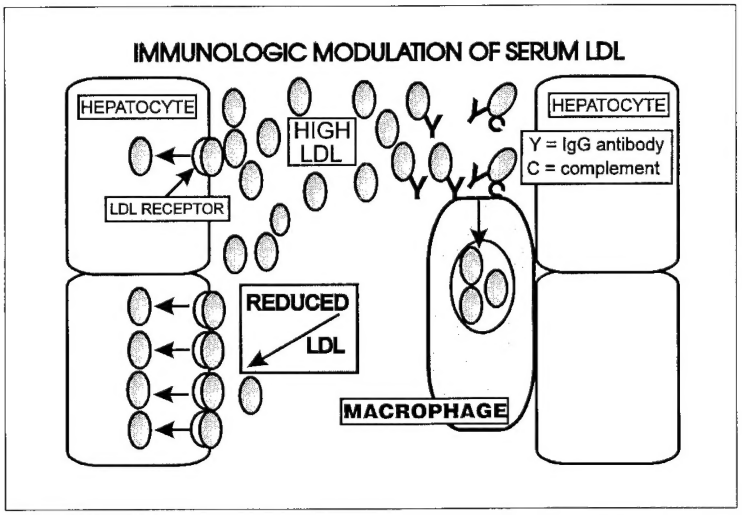

Fig. 4. Proposed mechanism of immunological modulation of diet-induced hypercholesterolemia.

in the serum was lost, presumably due to the binding of the antibodies to the VLDL/IDL that appeared in large amounts in the rabbit sera. Separate experiments demonstrated by ELISA that the antibodies did bind to purified rabbit VLDL/IDL obtained from serum of nonimmunized cholesterol-fed rabbits. Loss of the ability to detect antibodies at 6 weeks by ELISA did not occur with sera from rabbits fed a normal diet not supplemented with cholesterol (Fig. 2, inset). Similar patterns to those observed for IgG antibodies were also observed for IgM antibodies.

Although all the rabbits developed very high cholesterol levels (as high as 3,000 mg/dl in the nonimmunized animals at 12 weeks), the levels in the immunized animals were significantly and substantially lower than the nonimmunized animals (e.g., 1,770 mg/dl for immunized vs. 2,749 mg/dl for nonimmunized at 10 weeks, $p = 0.001$ by t-test).

The effect of immunization of rabbits against cholesterol on the subsequent development of aortic fatty streak lesions was determined. 40 animals were immunized monthly (0, 4, 8, and 12 weeks), and 5 weeks after completion of immunization the animals were placed on a 0.5% cholesterol diet for 12 weeks. The results showed markedly less sudanophilia in the immunized animals (Fig. 3). Although approximately 37% less sudanophilia was observed through the entire aortic surface, statistically significant less sudanophilia was demonstrated, 62 and 57% less respectively, in the descending thoracic aorta, and in the abdominal aorta (including the left and right renal arteries), both of which regions are magnified in Fig. 3.

Discussion

The results from the rabbit model demonstrate that immunization with a protein-free liposome formulation containing highly purified nonoxidized cholesterol and lipid A induces IgM and IgG antibodies that recognize both crystalline cholesterol and VLDL/IDL. The immunization procedure also provides prophylactic protection against diet-induced hypercholesterolemia and atherosclerosis. Figure 4 illustrates a mechanism that we believe can explain the experimental observations. We propose that the induced

antibodies can bind to cholesterol present in circulating LDL (or VLDL or IDL), thereby opsonizing the lipoproteins for removal by scavenger macrophages, principally Kupffer cells in the liver. The reduction of serum LDL then results in an upregulation in the number of LDL receptors [11], lowering the LDL still more and causing a further amplification of the beneficial effects.

Although it is true that the rabbit model might be considered somewhat unrealistic when compared with cholesterol levels that might occur in humans, the results suggest that immunization with liposomal cholesterol has tremendous potential potency in that it was able to lower the rabbit serum cholesterol level by as much as 979 mg/dl. This suggests that the immunization procedure might be an effective means of limiting the increases in serum cholesterol induced by diet in humans.

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